Dear editors and reviewers,

We thank the editors Inês Fragata and Raul Costa-Pereira, Bastien Castagneyrol and the anonymous reviewer for their work, comments and suggestions. We have tried to address all of them and to justify our choices and we respond to each comment below.

The revised manuscript by Migeon et al was assessed by two Reviewers and ourselves. We all agree that overall it has improved greatly from the previous version.

However, as pointed out by Reviewer 2 there is an important issue that largely reduces the power of several analyses and has not been addressed in the manuscript: the fact that the populations were assayed in different days adds a confounding effect to the population, which prevents comparisons between populations. In addition, this also severely impairs the correct estimation of the correlation between performance and the climatic variables. One possible angle is, as suggested by Reviewer 2 (in the first and second revision), to look at the intraspecific variation in the different populations and how it changes in response to drought stress. Even if the authors do not want to explore this angle, the fact that the populations were assayed in different days and the possible problems that can arise from it needs to be addressed in the results and discussion.

The main issue underlined by the editors and one reviewer is that the populations were assayed in different days and that they shouldn't be combined in the analysis. The experimental design, with separate assays of each population, prevents the data from being analyzed together. While this constraint is not ideal, it was simply impossible to run these trials simultaneously. Acknowledging this fact, we couldn't do the combined analysis proposed by Bastien Castagneyrol and we made separate analysis. To interpret our findings, we thus ran an independent analysis for each population. We present results graphically together for a visual comparison across models.

In addition, important information about the methods are missing. In particular, it is key to inform the date of collections, days when populations were assayed and how much time the populations spent in the laboratory (add these informations to the table). This allows the readers to acknowledge that time of collection/day/time in the laboratory can be factors at play in the results obtained.

All the information has been added in table 1.

In addition the material methods do not provide enough information to allow full reproducibility of the statistical analyses. Although we saw that the authors provided the R code and data, information should be also available in the methods section.

All the information regarding statistical analysis are now provided in the material section.

Figure 1 - This figure is a bit confusing, since there are three categories, the map should have only three colors (so that it is easy to see which populations belong in each category). This figure with the different colors could then be in supplementary material

Figure 1 has been redrawn with the same color scheme as the following figures. We propose to keep it here, as it offers a rapid view of the locations studied.

Line 85 - intraspecific variation has to be better articulated with the rest of the introduction.

We rephrased it and moved it to the bottom of the preceding paragraph so show the linkage more clearly.

Line 159: 163 - It is not clear what experiment these lines refer to.

We added, "*For each experiment,…*" to indicate that this was the case for all experiments done.

Line 282: 284 - It is not clear what was done in this analysis. Were all populations used in the same analyses? or one per population?

We rephrased the paragraph to be read, "*To examine the degree of phenotypic plasticity in the measured traits, we calculated the differences observed in life history traits between watering regimes. A small difference indicates little plasticity, while a large difference indicates substantial plasticity. We used linear regressions to explore how the climate of origin affected the magnitude of the plastic responses in the life history traits. Specifically, we evaluated the relationship between the reduction of development time, the increase in fecundity, the reduction in leaving rate, and the change in the progeny sex-ratio with drought stress and the 20 climatic variables (19 Bioclimatic variables + Global Aridity Index) of each collection location.*".

Review by Bastien Castagneyrol, 19 May 2022 19:13

The manuscript "Herbivore life histories are altered by drought stress in their host plants" presents the effect of drought on two fitness parameters of mites from different populations characterized by different water regimes. The authors show that mites perform better on water stress plants, and seemed to prefer them over well-watered plants. Importantly, they found that the effect of current drought was stronger in mites originating from wetter climate, demonstrating intraspecific variability (and possibly local adaptation) in response to drought.

The second version of the manuscript represents a clear improvement from the previous version. The authors addressed a fair amount of comments made by the three reviewers. In particular, the paper reads much better now, and several minor issues were clarified. However, I still need to be convinced by the modelling approach.

Inferences on the effect of drought on life history traits are based on a series of 12 repeated ANOVA, which is not recommended. This approach not only increases the risk of false discovery, but also prevents testing differences among populations. Yet, this is a key aspect of the paper. A possible alternative would consist in mixed-effect models in which population would be a random factor, crossed with plant identity, as there are two replicates (cotyledons) per plant.

While we agree that 12 ANOVA is not ideal, in regard of our experimental design we are following the comments from the following reviewer and the editor, who feel strongly that we not analyze data from separate experiments together.

1. I would appreciate to see proper hypotheses/predictions stated at the end of the introduction. Although I understand there are discrepancies in the literature, I believe this is possible here.

The last paragraph of the introduction has been changed and expanded and now reads, "*Here, we evaluate how intraspecific genetic variation modifies responses to drought stress, focusing on populations of the two spotted spider mite, T. urticae, sampled from locations in Europe with distinct climates. From the review of literature, we hypothesized that on drought-stressed plants relative to well-watered plants, females from all populations (1) will develop faster; (2) will have higher fecundity; (3) will decrease their leaving rate; (4) will give rise to a more female biased progeny. We also hypothesized that populations from drier regions, which are likely to more consistently experience drought-stressed plants, may exhibit less plasticity in these traits than populations from wetter more temperate regions that may experience only occasional drought stress. To test these hypotheses, we evaluated how mites from 12 sampling locations that differ in climate responded to drought-stress in their host plants. We examine the development time of females, their fecundity, the sex ratio of their progeny and the leaving rate on drought stressed and well-watered host plants. Finally, we relate the observed variation in these four life history traits to the climate of origin, and explore the potential adaptive value of different phenotypes.*".

2. I noticed the authors refrained from interpreting their results in terms of preference/performance of mites for/on drought-stressed plants. This is surely on purpose. Still, I had some difficulties to interpret the response of some life history traits. That's typically the case for development time and sex ratio. That would be great if the authors could link each life history traits with fitness/population performance (if it makes sense). I guess that reduced development time allows faster reproduction and escape from enemies (slow-growth-high-mortality hypothesis). Yet, if development time is time from egg to male and female emergence. I guess this encompasses embryonic development and larval development? Very little basic information on mite biology would be needed for non-mite people.

   We have added information in material section "*The embryonic (egg) development represents almost a half of the total duration of the development from egg laying to the adult hood. It is followed by three mobile stages interspersed by three immobile molting stages (Sabelis,1981)*" and interpretation on lines 466-467 which reads, *"…* which *reinforces the hypothesis of a general beneficial effect on the overall performance of mites on drought stressed plants.*".

3. L100 - is it correct to talk about "population fitness"? I thought it was defined at the level of individuals.

   Indeed, we have changed "fitness" for "*performance*"

4. L282-284 - In this paragraph, it is unclear to me what is the y-variable.

   The paragraph has been rephrased and now reads "*To examine the degree of phenotypic plasticity in the measured traits, we calculated the differences observed in life history traits between watering regimes. A small difference indicates little plasticity, while a large difference indicates substantial plasticity. We used linear regressions to explore how the climate of origin affected the magnitude of the plastic responses in the life history traits. Specifically, we evaluated the relationship between the reduction of development time, the increase in fecundity, the reduction in leaving rate, and the change in the progeny sex-ratio with drought stress and the 20 climatic variables (19 Bioclimatic variables + Global Aridity Index) of each collection location.*".

5. Figure 4 - Maybe consider adding cross-population means and SD?

   Figure 4 (renumbered figure 5) has been redrawn with these elements and another modification (same scale for young and old females).

6. L394-397 - This is an excellent summary! Maybe consider adding one opening sentence?

   Thank you, we have added an opening sentence that reads "*We have assessed 12 populations of mites originating from contrasted climatic condition, especially summer aridity index, combined with two water regimes.*".

7. Discussion: the mechanisms linking drought with increased fecundity are discussed. Should the reader assume that the same mechanisms (increase nutrient content) also explain faster development?

   That is our hypothesis and we have rephrased our analysis. Previous reports don't allow separation of fecundity and development time effects on increased performance. This section now reads, "*The increase of fecundity, along with shorter development time, confirms the previous reports of increased performance of T. urticae mites reared on drought-stressed plants (Chandler et al., 1979; Youngman and Barnes, 1986; Youngman et al., 1988; Ximénez-Embún et al., 2017a; Santamaria et al., 2018). Thus, the physiological changes that occur in drought-stressed plants are reliable changes. In tomato, these shifts in mite life history are likely linked to increased concentration of essential amino acids and free sugars (Ximénez-Embún et al., 2017a), which improved the nutritional value of drought-stressed plants. Similar changes may be occurring in drought-stressed bean plants.*".

8. Discussion (bis) - I would be curious to read how the authors interpret the fact that leaving rate was higher on well-watered plants. Is it because mites felt well on stressed plants, or because well-watered plants where somehow repulsive, or a combination of both. Likewise, there is no much discussion on the effect of drought on sex ratio, or about what it involves in terms of population adaptability.

   We propose an interpretation of the change of leaving rate at lines 486-490 that read, "*The higher leaving rate of the older females on well-watered plants may be linked to the lower performance of those same females on those plants with respect to fecundity and development time. However, in nature, the surrounding environment, mostly driven by the climate, may play a role by increasing or decreasing the risk of leaving a plant for a potential new plant.*". Regarding the sex-ratio we didn't observed enough consistency in the changes to be able to propose a hypothesis.

Review by anonymous reviewer, 19 May 2022 10:15

Review of the manuscript entitled "Herbivore life histories are altered by drought stress in their hosts plants", a second version of the pre-print "2021.10.21.465244".

First of all, I apologize to the authors and the recommenders for the delay in this review.

Overall, I found that the authors made a good effort to address most previous suggestions, making this version of the manuscript clearer and more streamlined.

Thank you

C1: However, in my point of view, the main issue which previously raised my concern (the fact that each population was tested in different moments) was not addressed or even acknowledged. Additionally, I believe that the authors have really interesting results, but I feel that they are underexplored.

We acknowledged this point, both in M&M, changed our analysis, and in the discussion. Please refer to the general response to the editors.

As I referred before, I would focus the discussion on the variability of responses observed within populations and why this is important for applied strategies to control this pest, in particular in the face of climate change. I also missed a more thorough link between the results from the different life history traits and the consequences that drought may have on the growth rate of the analysed populations and consequently on the interactions with their host plants. The way I see it, the discussion would gain a lot if sections 1 and 2 were merged. Note that I am not advertising against removing the discussion around the differences in climatic conditions from the collection locations, I would just frame it in the context of the differences observed within each population, instead of across populations. This suggestion is already incorporated in the results section, where the authors show that x out of y populations responded in a given away, but the link with the discussion is, in my view, important and missing.

We have preferred to keep the structure of the discussion. From our point of view, the separation of sections 1 and 2 allows a better way to analyze our results, but we agree that another way was possible.

Other than this I only have a few minor comments regarding this version of the manuscript:

C2: I believe that, in the title, the authors meant "life history traits" instead of "life histories" and "host plant" instead of "hosts plants".

We changed it. The new title is "*Climate of origin influences how a herbivorous mite responds to drought-stressed host plants.*".

C3, line 33: I would change "Thus, the climate in the area…" to "… , suggesting that the climate in the area…"

We changed it. The sentence now reads "*The mites from wet temperate climates exhibited greater plasticity between the two water regimes than mites originating from dryer and hot climates, suggesting that the climate in the area of origin influences mite response to drought.*".

C4: lines 74-76: The link between these two sentences is not very clear, could the authors please rephrase?

We rephrased and changed the sentences which now read "*These divergent results in how mites respond to drought-stressed plants might be explained in part by intraspecific variation among mite populations from different locations. In general, the degree to which different populations of a phytophagous arthropods differ in their responses to abiotic factors is poorly documented, but evidence suggests populations from different environments may often differ. For example, populations of a leaf beetle,* Diorhabda carinulata*, differ in the timing of diapause introduction across* *their North American range, enabling them to persist in environments with long cold winters south to desserts with milder winters (Bean et al. 2012).".*

C5 line 85: I would say "Interspecific genetic variation", otherwise intraspecific variation is the differences in response to… not a factor that can modify the response.

We changed and rephrased the sentence which now reads "*Intraspecific variation of this nature can be evident as fixed differences among populations from different environments, often due to adaptation to the local environment or to differences in phenotypic plasticity in response to the environment.*".

C6 line 124: I would remove the "and" after the reference (Migeon et al. 2019)

We removed it.

C7 line 125: This sentence is vague and does not have a reference. I believe that the authors are referring to the sample locations and not the countries as a hole. For example, the majority of Spain in figure 1 has a GAI equal or lower than that of Greece and Cyprus, so it is hard for me to consider it to have an "intermediate" climate. Also, this division is not in accordance with that made in figure S1 where the authors have a location in France and a location in the UK on the "medium climate" category.

We agree that this sentence was confusing and doesn't bring any information. We removed it. All the information is in the table and figure.

C8 line 420-422: the reference is missing.

We added the reference: Ximénez-Embún M.G., Castañera P., Ortego F. 2017a. Drought stress in tomato increases the performance of adapted and non-adapted strains of *Tetranychus urticae*. Journal of Insect Physiology, 96: 73-81.

C9 line 432-434: How do the author results reinforce the optimal balance hypothesis proposed by Alzate et al.? The authors did not measure longevity; the possible link between faster development and higher fecundity does not support the mentioned hypothesis and, as the authors state (lines 425-426), there is no link between early and late fecundity. Could the authors clarify?

We agree that this sentence represents an over-interpretation of our results and is not supported. We removed it.

C10 460-465: references are missing.

The references have been updated. The new sentences read "*These studies (Chen et al., 2020 and Bendena et al., 2008) tend then to support that local adaptation to diverse levels of aridity could shape mite responses allowing them to adjust feeding behavior in accordance with native local climatic conditions and nutritional quality of the host plants. However, the response to host plant drought stress is more complex than an increase of food consumption.*".